SYSTEM AND METHOD FOR SORTING CELLS

This Application is a continuation of U.S. patent application Ser. No. 11/536,492, filed on Sep. 28, 2006, which is a continuation U.S. patent application Ser. No. 10/378,109, filed Feb. 25, 2003, now U.S. Pat. No. 7,195,920, which is a divisional of U.S. patent application Ser. No. 09/511,959, filed on Feb. 23, 2000, now U.S. Pat. No. 6,524,860, which is a divisional of U.S. patent application Ser. No. 09/001,394, filed on Dec. 31, 1997, now U.S. Pat. No. 6,149,867, each of which are hereby incorporated by reference.

I. BACKGROUND OF THE INVENTION

This invention relates generally to the field of sex selection in mammalian offspring. It is especially relevant to the aspect of low dose artificial insemination for creating the desired sex of offspring. Particularly, the invention relates to systems for sorting sperm via flow cytometry for sex-specific and low dose efforts at artificial insemination or the like.

For ages it has been desired to select the sex of specific offspring. Beyond obvious psychological aspects, the actual sex selection of mammalian offspring has significant economic consequences when one considers its application to food producing animals such as cattle as well as celebrated trophy animals such as horses and the like. This great desire has resulted in a significant variety of efforts to achieve sex-selected offspring. Probably the effort which has appeared most likely to achieve the desired results has been efforts at sorting and selecting between X and Y sperm prior to insemination.

One of the challenges that effort at sorting X and Y sperm has faced is the large numbers of sperm involved. In natural insemination sperm are produced in some species by the billions; in artificial insemination less, but still significantly large numbers of sperm are used. For instance, artificial insemination techniques commonly use ten million to five hundred million sperm (depending on species). Thus a significant number of sperm are necessary even in an artificial insemination environment.

Many methods have been attempted to achieve the separation of X- and Y-chromosome bearing sperm. These methods have ranged from magnetic techniques such as appears disclosed in U.S. Pat. No. 4,276,139 to columnar techniques as appears disclosed in U.S. Pat. No. 5,514,537 to gravimetric techniques as discussed in U.S. Pat. No. 3,894,529, reissue Pat. No. 32350, U.S. Pat. Nos. 4,092,229, 4,067,965, and 4,155,831. Electrical properties have also been attempted as shown in U.S. Pat. No. 4,083,957 as well as a combination of electrical and gravimetric properties as discussed in U.S. Pat. Nos. 4,225,405, 4,698,142, and 4,749,458. Motility efforts have also been attempted as shown in U.S. Pat. Nos. 4,009,260 and 4,339,434. Chemical techniques such as those shown in U.S. Pat. Nos. 4,511,661 and 4,999,283 (involving monoclonal antibodies) and U.S. Pat. Nos. 5,021,244, 5,346,990, 5,439,362, and 5,660,997 (involving membrane proteins), and U.S. Pat. Nos. 3,687,803, 4,191,749, 4,448,767, and 4,680,258 (involving antibodies) as well as the addition of serum components as shown in U.S. Pat. No. 4,085,205. While each of these techniques has been presented as if to be highly efficient, in fact at present none of those techniques yield the desired level of sex preselection.

At present, the only quantitative technique used to achieve the separation of X- and Y-chromosome bearing sperm has been that involving individual discrimination and separation of the sperm through the techniques of flow cytometry. This technique appeared possible as a result of advances and discoveries involving the differential dye absorption of X- and Y-chromosome bearing sperm. This was discussed early in U.S. Pat. No. 4,362,246 and significantly expanded upon through the techniques disclosed by Lawrence Johnson in U.S. Pat. No. 5,135,759. The Johnson technique of utilizing flow cytometry to separate X- and Y-chromosome bearing sperm has been so significant an advancement that it has for the first time made the commercial separation of such sperm feasible. While still experimental, separation has been significantly enhanced through the utilization of high speed flow cytometers such as the MoFlo7 flow cytometer produced by Cytomation, Inc. and discussed in a variety of other patents including U.S. Pat. Nos. 5,150,313, 5,602,039, 5,602,349, and 5,643,796 as well as international PCT patent publication WO 96/12171. While the utilization of Cytomation's MoFlo® cytometers has permitted great increases in speed, and while these speed increases are particularly relevant given the high number of sperm often used, certain problems have still remained. In spite of the almost ten-fold advances in speed possible by the MoFlo® flow cytometer, shorter and shorter sorting times have been desired for several reasons. First, it has been discovered that as a practical matter, the sperm are time-critical cells. They loose their effectiveness the longer they remain unused. Second, the collection, sorting, and insemination timings has made speed an item of high commercial importance. Thus, the time critical nature of the sperm cells and the process has made speed an essential element in achieving high efficacy and success rates.

Other problems also exist ranging from the practical to the theoretical. On the practical side, it has been desired to achieve sex-sorted sperm samples using inexpensive disposable components and substances. Also on the expense side, it has been desired to be able to achieve sorting (as well as collection and insemination) in as efficient a labor event as possible. Thus, for commercial production and success in this field, improvements which might only represent an increase in efficiency may still be significant. Related to the practical aspect of expense, is the practical aspect of the delicateness and sensitivity of the entire process. In this regard, it has been desired to simplify the process and make it as procedurally robust as possible so that operator error or skill can play an ever decreasing role.

In addition to the delicateness of the process, it has always been known that the sperm themselves are extremely delicate cells. While this factor at first glance seems like it might be considered easily understood, in fact, the full extent of the cells' sensitivities have not yet been fully explored. In the context of flow cytometry in general, most sorted cells or particles have often been spherical or otherwise physically able to withstand a variety of abuses. This is not the case for sperm cells. In fact, as the present invention discloses, the processing through normal flow cytometer techniques may, in fact, be unacceptable for cytometric sorting of sperm cells in certain applications. The sensitivities range from dilution problems and the flow cytometer's inherent need to isolate and distinguish each cell individually as well as the pressure and other stresses which typical flow cytometry has, prior to the present invention, imposed upon the cells or other substances that it was sorting. This may also represent a unique factor for sperm cells because it appears that even though the sperm cell may appear to pass through the flow cytometer and be sorted with no visually discernable side-effects, in fact, the cells themselves may have been stressed to the point that they perform less than optimally in the insemination process. Thus, an interplay of factors seems involved and has raised